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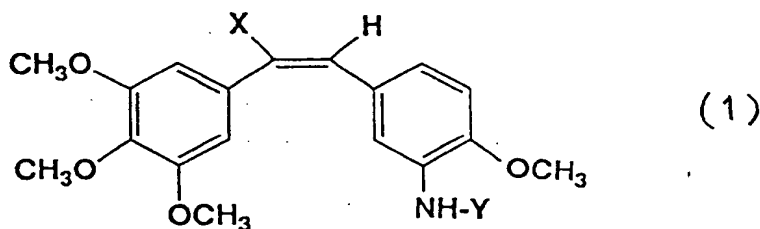
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(54) Stilbene derivatives and pharmaceutical compositions containing them

(57) Stilbene derivatives of the following formula (1) or their pharmaceutically acceptable salts are effective as carcinostatics and of low toxicity:



wherein X represents a hydrogen atom or a nitrile group, and Y represents an amino acid acyl group.

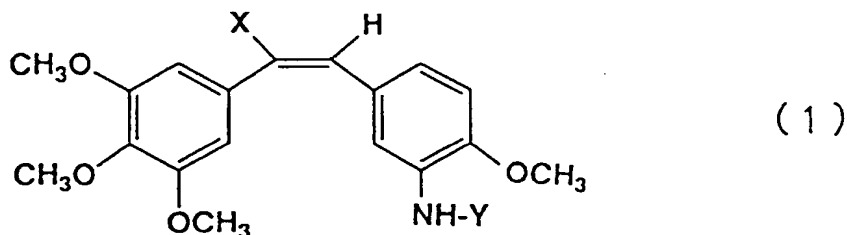
## Description

The present invention relates to cis-stilbene derivatives, to their use as pharmaceuticals and, in particular, to carcinostatics containing them as active ingredients.

It is known that combretastatins having cis-stilbene as their basic skeleton have strong mitosis inhibitory activity and strong cytotoxicity. However, since these compounds are barely soluble in water, they have not been put to practical use as medicines. Therefore, the development of the derivatives thereof have been studied (Molecular Pharmacology 34, Chii M. Lin et al., 200 - 206, 1988, J. Med. Chem., Mark Cushman et al., 1991, 34, 2579 - 2588, International Laid-Open Patent WO 92/16486, J. Med. Chem., Marck Cushman et al., 1992, 35, 2293 - 2306, International Laid-Open Patent WO 93/23357, J. Med. Chem., Mark Cushman et al., 1993, 36, 2817 - 2821, and Bioorg. Med. Chem. Let., Ryuichi Shirai et al., vol. 4, No. 5, pp. 699 - 704, 1994). Nevertheless, effective compounds have not yet been discovered.

The present invention relates to combretastatin derivatives which can be easily synthesized, which have low toxicity and which have pharmaceutical effect, and to provide carcinostatics containing them.

The present inventors have studied various stilbene derivatives which have an amino acid acyl group and screened carcinostatic compounds from them. Consequently, they have found that compounds of the following formula (1) have a remarkable carcinostatic effect and low toxicity in animal tests.



wherein X represents a hydrogen atom or a nitrile group, and Y represents an amino acid acyl group.

In formula (1), the amino acid acyl group is an acyl group derived from an amino acid. The amino acid includes  $\alpha$ -amino acids,  $\beta$ -amino acids and  $\gamma$ -amino acids. Preferable examples of the amino acid include glycine, alanine, leucine, serine, lysine, glutamic acid, aspartic acid, threonine, valine, isoleucine, ornithine, glutamine, asparagine, tyrosine, phenylalanine, cysteine, methionine, arginine,  $\beta$ -alanine, tryptophan, proline, and histidine. Especially, threonine and serine are preferable in terms of the pharmaceutical effects and safety. These amino acids may be L-isomers or D-isomers. The L-isomers are preferable.

Preferable examples of the compounds are as follows:

- (Z)-1-(3-amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene-glycineamide
- (Z)-1-(3-amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene-L-alanineamide
- (Z)-1-(3-amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene- $\beta$ -alanineamide
- (Z)-1-(3-amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene-L-leucineamide
- (Z)-1-(3-amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene-L-serineamide
- (Z)-1-(3-amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene-L-threonineamide
- (Z)-1-(3-amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene-L-valineamide
- (Z)-1-(3-amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene-L-isoleucineamide
- (Z)-1-(3-amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene-L-prolineamide
- (Z)-1-(3-amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene-L-methionineamide
- (Z)-1-(3-amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene-L-glutamineamide
- (Z)-1-(3-amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene-L-glutamylamide
- (Z)-1-(3-amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene-L-aspartylamide
- (Z)-1-(3-amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene-L-asparagineamide
- (Z)-1-(3-amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene-L-lysineamide
- (Z)-1-(3-amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene-L-histidineamide
- (Z)-1-(3-amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene-L-arginineamide
- (Z)-1-(3-amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene-L-cysteineamide
- (Z)-1-(3-amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene-L-tryptophanamide
- (Z)-1-(3-amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene-D-alanineamide
- (Z)-1-(3-amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene-D-leucineamide



were added thereto. The mixture was reacted at room temperature for 1 hour, and 100 ml of ether were added thereto. The resulting precipitate was collected by filtration. This precipitate was dissolved again in 30 ml of dioxane, and 0.5 ml of 2-M sodium hydroxide and 1.5 ml of water were added thereto. The mixture was reacted at room temperature for 1 hour. Subsequently, 20 ml of methanol were added to the reaction solution, and the mixture was poured into 250 ml of ether. The resulting precipitate was collected by filtration. The thus-filtered product was purified in small portions through medium-pressure liquid chromatography (ODS, mixture of water, acetonitrile and 12-N hydrochloric acid at a ratio of 75:-25:0.3). The thus-purified product was concentrated without being dried. When the amount of the solution reached approximately 50 ml, the solution was added to a mixture of ethyl acetate and ether at a ratio of 1:1, and precipitated. After the supernatant was discarded, 110 ml of acetonitrile and 350 ml of ether were added to the residue in this order. The resulting precipitate was filtered, washed with ether, and dried under reduced pressure to give 436 mg (0.838 mmols) in a yield of 33%.

$^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}$ )  $\delta$ : 2.120(q,  $J=7.0\text{Hz}$ , 2H), 2.468 (m, 2H), 3.735(s, 6H), 3.808(s, 3H), 3.888(s, 3H), 4.131(t,  $J=6.3\text{Hz}$ , 1H), 6.658(s, 2H), 6.995(d,  $J=8.6\text{Hz}$ , 1H), 7.143(d-d,  $J=2.2\text{Hz}$ , 8.6Hz, 1H), 7.349(s, 1H), 7.861(d,  $J=2.2\text{Hz}$ , 1H)

high-resolution mass spectrum, calculated - 470.1927, found - 470.1914

#### Example 16

##### Evaluation of cytotoxicity:

Mouse P388 leukemia cells were used as cancer cells, and a RPMI-1640 medium containing 5- $\mu\text{M}$  2-mercaptoethanol and 10 % fetal bovine serum was used in the incubation. The above-mentioned cells were inoculated on a 96-well microplate in an amount of  $1 \times 10^4$  cells/50  $\mu\text{l}$ /well, and an aqueous solution of a test compound (4  $\mu\text{g}/\text{ml}$ ) was added thereto in an amount of 25  $\mu\text{l}$ /well. The mixture was incubated at 37°C for 2 days. Then, the number of live cells were counted by the MTT method, and a dose-response curve was then prepared. A 50 % growth inhibitory concentration ( $\text{IC}_{50}$ ) given by the test compound was calculated according to the dose-response curve. The  $\text{IC}_{50}$  values of the compounds are tabulated below. Minimum doses which exert acute death immediately after injection are also shown in the table.

#### Example 17

##### Test for the pharmaceutical effect of mice:

Colon 26 which had been cloned subcutaneously in mice was cut with scissors, and implanted subcutaneously in mice by means of a trocar. One week later, the tumors were measured using calipers, and the volumes of the tumors were calculated. The mice were grouped (each group consisting of 3 mice). The test compound was dissolved with dimethylsulfoxide and diluted with 5 % Tween 80/saline. A 0.2 ml of the solution was injected intravenously once a day on Day 7, Day 11 and Day 15 after the implantation. On Day 21 after the implantation, the volumes of the tumors were measured. The volume of the tumor and the tumor growth inhibition rate (I.R.) were calculated using the following expressions.

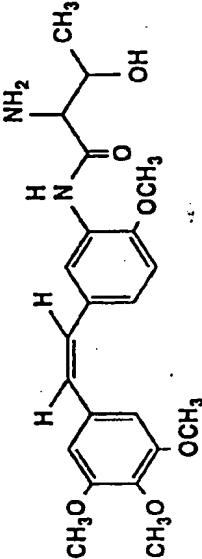
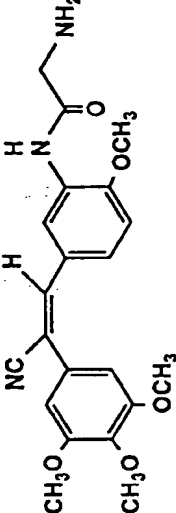
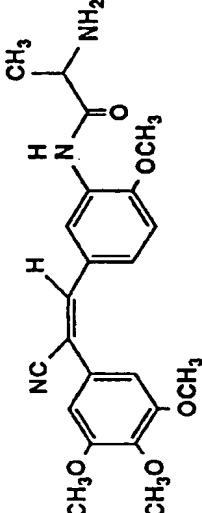
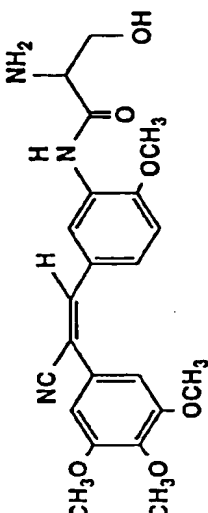
$$\text{Volume of tumor} = \frac{(\text{short diameter})^2 \times (\text{long diameter})}{2}$$

$$\text{I.R. (\%)} = \frac{1 - (\text{average tumor volume of agent-administered group})}{(\text{average tumor volume of control group})} \times 100$$

Compound Name	Formula	in vitro IC <sub>50</sub> (ng/ml)	in vivo a) I.R. (%)	Toxic Dose (mg/kg) b)
(Z)-1-(3-amino-4-methoxy phenyl)-2-(3,4,5-trimethoxy phenyl)-ethene-L -glycine amide		2.0	33.3 (40mg/kg)	80
(Z)-1-(3-amino-4-methoxy phenyl)-2-(3,4,5-trimethoxy phenyl)-ethene-L -alanine amide		2.0	51.9 (40mg/kg)	80
(Z)-1-(3-amino-4-methoxy phenyl)-2-(3,4,5-trimethoxy phenyl)-ethene-L -leucine amide		6.0	50.9 (40mg/kg)	40
(Z)-1-(3-amino-4-methoxy phenyl)-2-(3,4,5-trimethoxy phenyl)-ethene-L -serine amide		4.0	72.9 (80mg/kg)	160

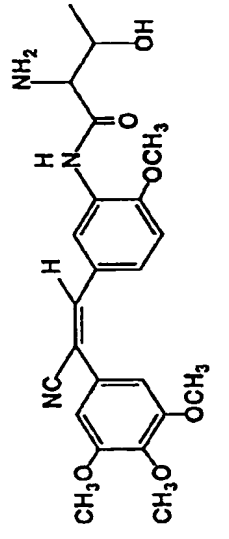
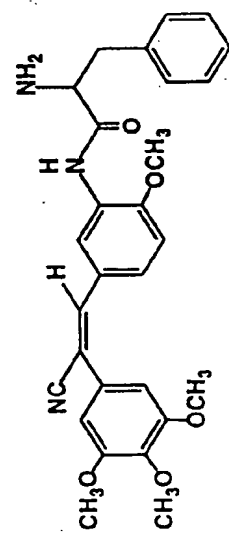
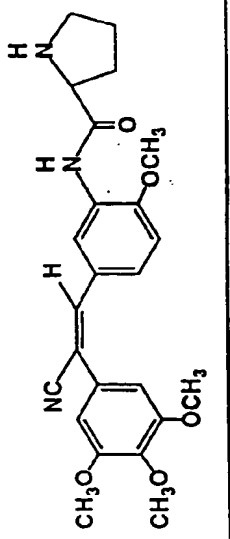
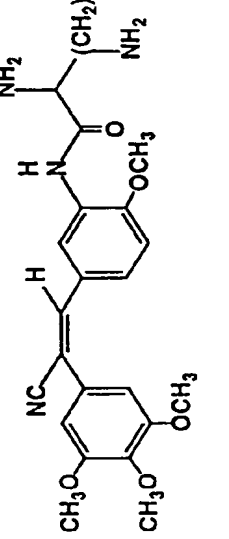
a) Administered once a day on day 7, day 11 and day 15 intravenously.

b) A minimum dose which show death immediately after injection.

Compound Name	Formula	in vitro IC <sub>50</sub> (ng/ml)	in vivo a) I.R. (%)	Toxic Dose (mg/kg) b)
(Z)-1-(3-amino-4-methoxy phenyl)-2-(3,4,5-trimethoxy phenyl)-ethene-L -threonine amide		6.0	62.2 (80mg/kg)	160
(E)-3-(3-amino-4-methoxy phenyl)-2-(3,4,5-trimethoxy phenyl)-prop-ene-nitrile -L-glycine amide		3.0	85.7 (20mg/kg)	40
(E)-3-(3-amino-4-methoxy phenyl)-2-(3,4,5-trimethoxy phenyl)-prop-ene-nitrile -L-alanine amide		0.5	71.0 (20mg/kg)	80
(E)-3-(3-amino-4-methoxy phenyl)-2-(3,4,5-trimethoxy phenyl)-prop-ene-nitrile -L-serine amide		2.0	75.0 (80mg/kg)	160

a) Administered once a day on day 7, day 11 and day 15 intravenously.

b) A minimum dose which show death immediately after injection.

Compound Name	Formula	in vitro IC <sub>50</sub> (ng/ml)	in vivo a) I.R. (%)	Toxic Dose (mg/kg) b)
(E)-3-(3-amino-4-methoxy phenyl)-2-(3,4,5-trimethoxy phenyl)-prop-ene-nitrile -L-threonine amide		6.0	67.0 (40mg/kg)	320
(E)-3-(3-amino-4-methoxy phenyl)-2-(3,4,5-trimethoxy phenyl)-prop-ene-nitrile -L-phenylalanine amide		5.0	76.7 (40mg/kg)	80
(E)-3-(3-amino-4-methoxy phenyl)-2-(3,4,5-trimethoxy phenyl)-prop-ene-nitrile -L-proline amide		200	68.9 (40mg/kg)	40
(E)-3-(3-amino-4-methoxy phenyl)-2-(3,4,5-trimethoxy phenyl)-prop-ene-nitrile -L-ornithine amide		50.0	48.9 (10mg/kg)	N. D.

a) Administered once a day on day 7, day 11 and day 15 intravenously.

b) A minimum dose which show death immediately after injection.